Measuring the Thermodynamics of Enzyme Reactions to Enable Better Manufacturing of Biotechnology Products

NIST is using its expertise in enzyme thermodynamics measurement to conduct studies to obtain quantitative data on two key enzymes used in bioprocess manufacturing of high purity amino acids. These measurement data can be used to enable the innovation of better enzymes for bio-process engineering of valuable biotechnology products, including: antibiotics, hormones, pesticides and artificial sweeteners.

Y. B. Tewari, B. E. Lang, and R. N. Goldberg (831)

The enzymes **D**-hydantoinase and *N*-carbamoyl-**D**-amino acid hydrolase are used for the commercial production of optically pure amino acids. These optically pure amino acids are, in turn, widely used to produce a wide variety of biotechnology products that include semi-synthetic antibiotics, active peptides, pharmaceuticals, hormones, pesticides, and sweeteners. Interest in these enzymes is also increasing as evidenced by numerous molecular biology studies involving the enzymes and the reactions that the enzymes catalyze. Following the suggestion of an industrial contact who pointed out to us the absence of any thermodynamic investigations, we initiated a series of equilibrium and calorimetric measurements on these reactions with the aim of providing reliable thermodynamic data that could enhance the efficient bioprocess engineering of the reactions catalyzed by these enzymes.

NIST impacts the biotechnology industry by applying its expertise in thermodynamics for measuring reaction characteristics of important industrial enzymes. Development of these reference data enables a better understanding of the fundamental approach for designing these economically important biomolecules.

We used HPLC, calorimetry, and equilibrium modeling calculations to study several representative reactions catalyzed by these enzymes. For the reactions catalyzed by **D**-hydantoinase, we were able to measure values of the apparent equilibrium constants *K*'. However, the reactions catalyzed by *N*-carbamoyl-**D**-amino acid hydrolase proceeded to completion and equilibrium measurements were not possible. Calorimetric measurements were performed on several individual and combined reactions. In those cases where measurements were not possible, we used property values for structurally similar reactions to provide estimates for the needed property values. The thermodynamic results obtained in this study provide quantitative data that can be used for the efficient bioprocess engineering of these enzyme-catalyzed reactions. The results obtained in this investigation are the first to be reported in the literature on the thermodynamics of these reactions.

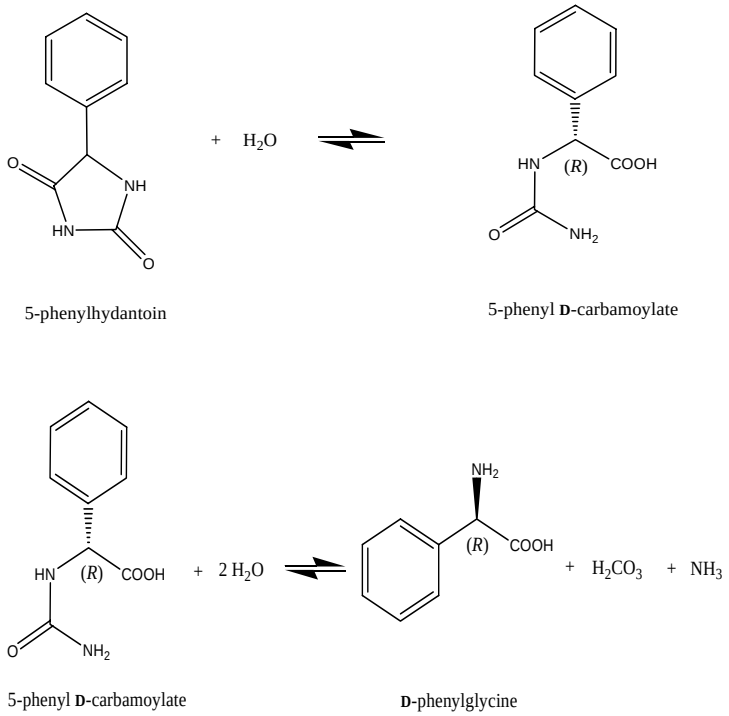

Figure caption: ***Representative reactions catalyzed, respectfully, by D-hydantoinase and by N-carbamoyl-D-amino acid hydrolase.***

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